

Application No.: 10/727,292

Docket No.: JCLA12308-R

In the Specification:

Please amend paragraphs [0001], [0005], [0007], [0009], [0016], [0021], [0022] and [0031]-[0036] as follows.

[0001] The present invention relates to a method for producing a soluble composition containing ~~soluble~~ isoflavones.

[0005] In view of the foregoing, one object of this invention is to provide a method for producing a soluble composition containing ~~soluble~~ isoflavones, wherein soybean materials are used as raw materials and isoflavones are extracted in their natural forms without addition of solubilizing agents and chemical modification. The soluble composition containing ~~soluble~~ isoflavones has high solubility over a wide pH range, and has good long-term stability under refrigeratory preservation.

[0007] That is, this invention is directed to 1) a method for producing a soluble composition containing ~~soluble~~ isoflavones from a soybean extract liquid, featuring with removal of insoluble materials from the soybean extract liquid that has a pH value adjusted to 2-7 and a temperature adjusted to 0-17°C; 2) a method for producing a soluble composition containing ~~soluble~~ isoflavones according to 1), wherein the amount of isoflavones in total solid content of the soybean extract liquid is 0.2-20wt%, the crude protein content is 30wt% or less, and the amount of lipids is 4wt% or less; 3) a method for producing a soluble composition containing ~~soluble~~ isoflavones according to 1) or 2), wherein the process of preparing the soybean extract liquid includes adjusting the pH value to 5.5-7 without a protease treatment; and 4) a method for producing a soluble

Application No.: 10/727,292

Docket No.: JCLA12308-R

composition containing ~~soluble~~ isoflavones according to 1) or 2), wherein the process of preparing the soybean extract liquid includes a step of adjusting the pH value to satisfy the equation " $2 \leq \text{pH} < 5.5$ " and a protease treatment.

[0009] The present invention is specifically described as follows. The soluble composition containing ~~soluble~~ isoflavones of this invention is prepared from a soybean extract liquid, which is obtained by treating soybean materials with an extracting solvent.

[0016] Thereafter, the pH value of the soybean extract liquid obtained as above is adjusted to 2-7, and the soybean extract liquid is cooled to 0-17°C, preferably 0-10°C, and kept at the same temperature for 10 minutes or more, preferably 30 minutes or more, whereby some low-temperature insoluble materials are formed. If the pH value is adjusted smaller than 2, the isoflavones may self-decompose. On the other hand, the solubility of isoflavones gets low under a neutral to acidic condition having a pH value larger than 7. The pH value can be adjusted to any value within the range of 2-7, and may be adjusted to be equal to or smaller than that of the product into which the composition containing isoflavones will be added. When the cooling temperature is higher than 17°C, precipitation or cloudiness occurs even if the pH value of the soybean extract liquid is adjusted to be equal to or smaller than that of the product. The acid for adjusting the pH value to 2-7 can be any one among the inorganic acids and the organic acids that are usually added in food. The applicable acids include hydrochloric acid, sulfuric acid, phosphoric acid, acetic acid, malic acid, tartaric acid, citric acid and ascorbic acid, etc., wherein hydrochloric acid is particularly preferable. Subsequently, the insoluble

Application No.: 10/727,292

Docket No.: JCLA12308-R

materials are removed with, for example, filtration or centrifugal separation, and a soluble composition containing ~~soluble~~ isoflavones is obtained thereby. Generally, when proteins and other components get insoluble and precipitate because of cooling effect, the isoflavones contained in the soybean extract liquid easily co-precipitate with them. However, since the raw material used in this invention is not subjected to a physical treatment like pulverization, exudation of proteins and other components from the raw material can be inhibited to prevent co-precipitation. Therefore, the recovery ratio of isoflavones is not lowered.

[0021] The soluble composition containing ~~soluble~~ isoflavones obtained as above can be directly used after the concentration thereof is properly adjusted. The composition may also be used in the form of condensed extract after being neutralized as required and condensed, or in the form of dry matter after a further drying process like a freeze-drying process or a spray-drying process, etc. Moreover, if required, a further process like a purification process using an adsorbent or a separation process using a solvent can be performed to produce a soluble composition containing ~~soluble~~ isoflavones of higher purity. The adsorbent used in the purification process can be of polystyrene type, methacryl type or ODS type, etc, and the solvent used in the separation process can be butanol or the like.

[0022] When the amount of isoflavones in total solid content is 0.2-10wt% in the composition and the composition is used in a product having a pH value larger than that on removing insoluble materials, cloudiness or precipitation does not occur even if the

Application No.: 10/727,292**Docket No.: JCLA12308-R**

product is refrigerated under 10°C. That is, the composition shows high stability and a good solubility. The solubility of pure isoflavone in water is merely 0.002-0.003g, while that of the isoflavones in the soluble composition containing ~~soluble~~ isoflavones of this invention is 0.3g or more (0.3-100g). That is, it is possible to dissolve a large amount of isoflavones that is 100 or more times the amount in the prior art. Therefore, all neutral to weak acidic food products, such as neutral drinks like tea drinks or weak acidic drinks like coffee as well as ice cream and dessert, etc., can be applied with high concentrations of isoflavones without loss of transparency. In addition, the solubility in this invention is defined as the maximal amount of a solute dissolved in a solvent of 100g under 25°C.

[0031] 100g of whole soybeans from the America, in which the amount of isoflavones is 0.2wt%, is added with 500ml of water in a pre-treatment, wherein the soybeans contact with water under 20°C for 2 hours. The liquid part is then removed with filtration. Next, the residue is added with 500ml of water to implement an extraction process under 98°C for 20 minutes, and then an extract liquid is obtained with filtration. The residue is further added with 500ml of water to implement another extraction process under 98°C for 20 minutes, and then another extract liquid is obtained with the same operation. The two extract liquids are mixed into a soybean extract liquid. In the soybean extract liquid, the amount of isoflavone in total solid content is 1.3wt%, the crude protein content is 24wt%, and the lipid content is 1.0wt%. The soybean extract liquid is cooled to 10°C and kept at the same temperature for 30 minutes, while the pH value is 6.5 at the moment. Next, the insoluble materials are removed with centrifugal separation, and the soybean

Application No.: 10/727,292

Docket No.: JCLA12308-R

extract liquid is freeze-dried into powder. Thus, a soluble composition of 9.4g containing ~~soluble~~ isoflavones in an amount of 1.34wt% is obtained. In the example, the recovery ratio of isoflavones from the whole soybeans is 63.0%.

[0032] 100g of soybean hypocotyls from the America, in which the amount of isoflavones is 1.6wt%, is dry-heated under a hot blast of 140°C for 20 minutes using a gas roaster. After the treatment, the soybean hypocotyls is added with 500ml of water in a pre-treatment, wherein the soybean hypocotyls contact with water under 20°C for 2 hours. The liquid part is then removed with filtration. Next, the residue is added with 500ml of water to implement an extraction process under 98°C for 20 minutes, and then an extract liquid is obtained with filtration. The residue is further added with 500ml of water to implement another extraction process under 98°C for 20 minutes, and then another extract liquid is obtained with the same operation. The two extract liquids are mixed into a soybean extract liquid. In the soybean extract liquid, the amount of isoflavones in total solid content is 6.0wt%, the crude protein content is 22wt%, and the lipid content is 0.5wt%. The soybean extract liquid is cooled to 10°C and kept at the same temperature for 30 minutes, while the pH value is 6.5 at the moment. Next, the insoluble materials are removed with centrifugal separation, and the soybean extract liquid is freeze-dried into powder. Thus, a soluble composition of 18.7g containing ~~soluble~~ isoflavones in an amount of 6.06wt% is obtained. In the example, the recovery ratio of isoflavones from the soybean hypocotyls is 70.8%.

Application No.: 10/727,292

Docket No.: JCLA12308-R

[0033] 100g of whole soybeans from the America, in which the amount of isoflavones is 0.2wt%, is added with 500ml of water in a pre-treatment, wherein the soybeans contact with water under 20°C for 2 hours. The liquid part is then removed with filtration. Next, the residue is added with 500ml of water to implement an extraction process under 98°C for 20 minutes, and then an extract liquid is obtained with filtration. The residue is further added with 500ml of water to implement another extraction process under 98°C for 20 minutes, and then another extract liquid is obtained with the same operation. The two extract liquids are mixed into a soybean extract liquid. In the soybean extract liquid, the amount of isoflavone in total solid content is 1.3wt%, the crude protein content is 24wt%, the lipid content is 1.0wt%, and the pH value is 6.5 at the moment. Next, a neutral protease from *Bacillus subtilis* (Orientase 90N, produced by Hankyu Kyohei Bussan Inc.) is added in an amount of 0.9wt% relative to the total solid content, and the reaction is carried out under 50°C for 1 hour. Next, the soybean extract liquid is heated under 80°C for 30 minutes to deactivate the enzyme, and the pH value is adjusted to 3.5 by adding HCl_(aq). The soybean extract liquid is cooled to 10°C and placed still for 30 minutes. Next, the insoluble materials are removed with centrifugal separation, and the soybean extract liquid is neutralized by adding NaOH to have a pH value of 6.5. The soybean extract liquid is then freeze-dried into powder, and a soluble composition of 8.8g containing ~~soluble~~ isoflavones in an amount of 1.40wt% is obtained. In the example, the recovery ratio of isoflavones from the whole soybeans is 61.6%.

Application No.: 10/727,292

Docket No.: JCLA12308-R

[0034] 100g of soybean hypocotyls as obtained in Example 2, in which the amount of isoflavones is 1.6wt%, is added with 500ml of water in a pre-treatment, wherein the soybeans contact with water under 20°C for 2 hours. The liquid part is then removed with filtration. Next, the residue is added with 500ml of water to implement an extraction process under 98°C for 20 minutes, and then an extract liquid is obtained with filtration. The residue is further added with 500ml of water to implement another extraction process under 98°C for 20 minutes, and then another extract liquid is obtained with the same operation. The two extract liquids are mixed into a soybean extract liquid. In the soybean extract liquid, the amount of isoflavone in total solid content is 6.0wt%, the crude protein content is 22wt%, the lipid content is 0.5wt%, and the pH value is 6.5 at the moment. Next, a neutral protease from *Bacillus subtilis* (Orientase 90N, produced by Hankyu Kyoei Bussan Inc.) is added in an amount of 0.9wt% relative to the total solid content, and the reaction is carried out under 50°C for 1 hour. Next, the soybean extract liquid is heated under 80°C for 30 minutes to deactivate the enzyme, and the pH value is adjusted to 3.5 by adding HCl_(aq). The soybean extract liquid is cooled to 10°C and placed still for 30 minutes. Next, the insoluble materials are removed with centrifugal separation, and the soybean extract liquid is neutralized by adding NaOH to have a pH value of 6.5. The soybean extract liquid is then freeze-dried into powder, and a soluble composition of 15.5g containing ~~soluble~~ isoflavones in an amount of 6.32wt% is obtained. In the example, the recovery ratio of isoflavones from the soybean hypocotyls is 61.2%.

[0035] A soybean extract liquid as obtained in Example 4 is added with HCl_(aq) to have a

Application No.: 10/727,292

Docket No.: JCLA12308-R

pH value of 3.5 without the protease treatment, and is cooled to 10°C and placed still for 30 minutes. After the insoluble materials are removed with centrifugal separation, the soybean extract liquid is neutralized by adding NaOH to have a pH value of 6.5. The soybean extract liquid is then freeze-dried into powder, and a soluble composition of 10.3g containing ~~soluble~~ isoflavones in an amount of 4.72wt% is obtained. In the example, the recovery ratio of isoflavones from the soybean hypocotyls is 30.4%. Though the solubility of the composition in water is relatively high, the recovery ratio of isoflavones gets low since a part of the isoflavones are removed together with the insoluble materials during the removal step under acidic condition.

[0036] 10g of the soluble composition containing ~~soluble~~ isoflavones as obtained in Example 4 is dissolved in 100ml of water, and then the solution is conducted through a column (ϕ 2.5cm×20cm) packed with 100ml of an activated synthetic adsorbent of styrenedivinylbenzene type (Diaion HP-20, produced by Mitsubishi Chemical Corporation) in a flow rate of 100ml/hr. Next, the adsorbent is washed with 200ml of water and 200ml of 20% ethanol in sequence to remove impurities, and then the target materials are eluted with 300ml of 70% ethanol. After being condensed under low pressure to remove ethanol, the solution is freeze-dried into a soluble composition of 1.5g containing ~~soluble~~ isoflavones in an amount of 26.2wt%.